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## **PENETRATION OF ORGANIC CHEMICALS THROUGH THE HAIR FOLLICLES AND THE EPIDERMIS**

**BY**

**R. T. TREGEAR**

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PENETRATION OF ORGANIC CHEMICALS THROUGH THE  
HAIR FOLLICLES AND THE EPIDERMIS

By

R. T. TREGEAR

INTRODUCTION

The relative importance of the appendages and the epidermis in the passage of chemicals into the skin has been debated for many years (1, 2). Conclusions on the relative importance of the epidermis and the pilosebaceous apparatus have been based principally on the histological localisation of the penetrant (3, 4), but such observations show only the concentration in the tissue and not the rate at which the penetrant diffuses through it. Only direct measurements of the penetration rates through each of the two structures separately would determine their relative importance, and this has been attempted in the present experiments.

METHODS

A young pig was anaesthetised with dial and urethane and the ventral part of one flank was clipped and washed. Three to five circular areas, each 1" in diameter, were marked out on the skin in a pattern corresponding to the holes in a metal plate (Fig. 1). 2-3  $\mu$ g drops of P32-labelled VX (20-40 mC/g) dyed blue with an anthraquinone (Grasol blue LS) were applied to these skin areas with a fine pointer, 20-30 drops on each area; the drops were placed either over hair follicles or on the epidermis between them (Fig. 2). The epidermally applied drops usually spread to cover a few hair follicles while drops deliberately placed on follicles spread to a small extent over the adjoining epidermis (Fig. 2); the number of hair follicles covered by blue dye was counted at the end of the experiment.

The skin areas were covered with mica under the plate, sealed to the skin with adhesive tape and each area in turn was exposed to a Geiger counter. These measurements were continued for 5-6 hours. The regression

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equation of counting rate (radioactivity) with time (Fig.3) was calculated, and the penetration rate per unit mass of VX applied was derived from the formula:

$$\text{Penetration rate (g/min/g applied)} = \frac{\text{Decrease in counting rate per unit time}}{\text{Original counting rate}} = \frac{dC/dt}{C_0}$$

This experimental method has already been described and its validity discussed(5).

At the end of the experiment an autoradiograph of the VX in each area was taken by pressing an X-ray film into contact with the mica covering it. Autoradiographs were also taken of VX P32-saturated filter paper discs of known diameter, and the width of the halo produced by the long-range  $\beta$ -particles from the P32 measured; this was found to be 0.08mm. The area of skin covered by each drop of VX was taken as that of the autoradiograph less that of the halo. A photograph of each skin area was accurately superimposed on the corresponding autoradiograph; thus the number of hair follicles covered by P32 at the end of the experiment could be counted. Finally each skin area was dissected out, digested in nitric acid and its P32 content determined. From this and from the observed penetration rate the total VX which had been applied to the area was calculated.

The average length and diameter of the hair follicles in the ventral part of the flank were measured. Similar measurements were made on skin from various other species, and the number of hairs per unit area of skin surface in each was counted using a dissecting microscope. From these measurements the total follicular epithelial area per unit of skin surface was calculated.

RESULTS

1. Penetration and the hair follicles

A few follicles were always covered even by the most careful epidermal application (Table 1). Many more follicles were filled, however, when the drops were deliberately applied over them (Table 2,4).

TABLE 1

Hair follicles filled with VX after epidermal application

As indicated by:

dye 2.6/ exp. area

P32 2.4/ exp. area

Each value is the mean of 9 observations

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TABLE 2

The results of epidermal (E) and follicular (F) application of VX

A. Number of hair follicles filled with VX		B. Penetration rate of VX (g/min x 10 <sup>-9</sup> ) per exp. area		C. Penetration rate per unit area (g/min/cm <sup>2</sup> x 10 <sup>-9</sup> )					
E	F	E	F	E	F				
8.0	19.0	20	22	62	84				
2.7	18.0	17	22	63	116				
4.7	19.5	20	23	54	71				
1.5	19.5	10	16	30	67				
1.5	25	12	14	30	38				
0.5	21	6	4	-	-				
Means <sup>x</sup> 3.4	20	15	19	49	80				
Difference <sup>x</sup> 15.6		3.2		31					
% confidence 13.6-17.6 limits		0.5-5.9		17-45					
Each value is the mean for a single experiment, derived from 1-3 skin areas.									
*The mean and difference values were obtained statistically as explained in the appendix and therefore do not correspond exactly with the values in the table.									

Although the penetration rate was slightly greater with the follicular than with the epidermal application (Table 2B) this difference was only a small fraction of what it would have been if the rate had been proportional to the number of hairs filled with VX (Fig.4); the hair follicles, therefore, were not the most important route of penetration.

Nevertheless, the hair follicles did have a definite effect on the penetration rate. Although follicular application produced only a slightly greater overall penetration rate than epidermal application (Table 2B) the penetration rate per unit area of skin covered by VX was much increased.

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(Table 20). This showed that the hair follicles were considerably more permeable than epidermis, area for area.

## 2. The relative penetrability of hair follicles and epidermis

Since VX appeared to penetrate through both these routes the following formula was applied:

where  $n$  = number of hair follicles filled with VX

A = area of epidermis covered with VX ( $\text{mm}^2$ )

$p_f$  = penetration rate per follicle (g/min/follicle)

$p_e$  = penetration rate per unit area of epidermis (g/min/mm<sup>2</sup>)

R = overall penetration rate (g/min)

From this formula, and using the statistical technique described in Appendix B, values of the ratio  $p_f/p_e$  were obtained from each experiment in turn (Table 3); the range of the mean shows that a hair follicle could have been equivalent to anything between  $2/3$  and  $3\frac{1}{2}$   $\text{mm}^2$  of epidermis.

TABLE 3

The ratio of the penetration rate per hair follicle and per sq.mm of epidermis

Experiment	$p_f/p_e$ (mm <sup>2</sup> )
1	2.05
2	3.58
3	1.21
4	1.03
5	0.66
Geometric mean	1.43
% confidence limits	0.63 - 3.25

These figures were obtained by a statistical method described in Appendix B.

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Histological measurement showed that the cross-sectional area of a hair follicle ( $a_1$ ) was  $0.019 \text{ mm}^2$ ; it follows that the follicles were considerably more permeable than an equivalent area of epidermis:

$$\frac{\text{Follicular penetration/unit cross-sect. area}}{\text{Epidermal penetration/unit area}} = \frac{1}{a_1} \frac{p_f}{p_e}$$
$$= 75 \text{ (Range 33-170)}$$

On the other hand the mean epithelial area inside a hair follicle ( $a_2$ ) was much greater,  $1.05 \text{ mm}^2$ , so that the penetration per unit epithelial area within the follicle did not significantly differ from that of the epidermis:

$$\frac{\text{Follicular penetration/unit epithelial area}}{\text{Epidermal penetration/unit area}} = \frac{1}{a_2} \frac{p_f}{p_e}$$
$$= 1.4 \text{ (Range 0.6-3.2)}$$

3. The area of intra-follicular epithelium in different species

The cross-sectional area of the follicles was calculated from their mean diameter and the area of the epithelial lining was calculated from their diameter and length, on the assumption that they were approximately cylindrical. From these values and the number of hairs per unit surface area, the total areas of skin surface taken up by the follicles ( $A_1$ ) and of stratified epithelium lining ( $A_2$ ) were determined. In hairy animals there was considerably more epithelium below the skin surface than upon it, although only a small fraction of the visible surface was taken up by hairs (Table 4).

TABLE 4

Fraction of skin surface covered by hair follicles ( $A_1$ ) and epithelial area within follicles per unit skin area ( $A_2$ ):  $(\text{cm}^2/\text{cm}^2)$

Species & site	$A_1$	$A_2$
Man, dorsal forearm	0.0015	0.07
Pig, lower flank	0.0024	0.13
Rat, belly	0.041	2.3
Rabbit, back	0.018	12.0

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DISCUSSION

The present experiments were designed to find out whether penetration is chiefly through hair follicles or through epidermis. Deliberate efforts were made to encourage follicular passage, but there was only a slight increase in penetration when the number of hair follicles covered was increased by a factor of five or more (Fig. 4); in these experiments, therefore, only a small fraction of the penetrant passed through the follicles. However, when the penetration was expressed in terms of the area of skin covered the hair follicles in the pig were found to be 30-160 times as permeable as an equivalent area of epidermis.

When a large drop is applied it covers both hair follicles and epidermis, so that the fraction of skin penetration which occurs via the hair follicles can be calculated from the total cross-sectional area of follicles per unit area of skin ( $A_f$ ). On the assumption that the mean of 75 found in the pig for the ratio of follicular to epidermal penetrability holds in other species this fraction is given by the expression:

$$\frac{75A_f}{1 + 74A_f} \dots \dots \dots \quad (2)$$

From this the values in Table 5 (Col. A) have been calculated; although these are only approximate they demonstrate the relative unimportance of the hair follicles in man and their greater importance in the hairy animals.

TABLE 5

Fraction of total penetration which passes through the hair follicles, calculated from equations 2 (Column A) and 3 (Column B)

Species & Site	Follicular penetration	
	A	B
Man, dorsal forearm	0.11	0.06
Pig, lower flank	0.15	0.12
Rat, belly	0.58	0.70
Rabbit, back	0.76	0.92

There are two possible routes of penetration in the follicle, the sebaceous glands and the stratified epithelium lining the hair shaft. Although fat-soluble penetrants have been observed within the sebaceous glands (3,6) it seems unlikely that much passes from them into the circulation, for such substances would be preferentially absorbed by the

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gland cells. The results reported here show that when the epithelial lining of the follicles is considered the penetration per unit area is approximately the same as that for the epithelium on the skin surface; in other words, if the sebaceous gland penetration is negligible all stratified epithelium, whether inside or outside the follicles, appears to have the same penetrability.

On this basis the fraction of the penetrant which penetrates via the hair follicles may be calculated from the area of epithelium lining the follicles ( $A_2$ ) from the expression

The values calculated this way are also shown in Table 5, and do not differ markedly from those calculated by the first method.

In man another possible route is penetration through the sweat glands. Palmar skin has been found to be considerably less penetrable than that of other regions (6,7,8), although the sweat glands are several times as numerous there as on the arm, thigh or trunk (9). If the amount of penetration per gland is calculated on the assumption that all the palmar penetration is by this route, it would only account for a small fraction of the higher rate of penetration with lower gland density found elsewhere. Penetration via the sweat glands is therefore unlikely to be important in regions other than the palms and soles.

If the sweat and sebaceous glands are unimportant and all the epithelium has the same penetrability, it is correct to express the penetration of skin in terms of the total epithelial area rather than the skin surface area. When the figures previously obtained for VX are compared in this way they all lie within a comparatively narrow range (Table 6), which suggests that all stratified epithelia present comparable barriers to penetration.

TABLE 6

Penetration of  $VX$  expressed per unit area of skin surface (A) or per unit area of the total epithelial surface (B)

Species	Penetration rate (g/cm <sup>2</sup> /min x 10 <sup>-6</sup> )	
	A	B
Man, back	0.47	0.44
Pig, flank	0.78	0.69
Rat, belly	2.5-7.3	0.7-2.1
Rabbit, back	4.7	0.36

The values in column A were taken from ref.10 & the epithelial areas from Table 4.

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Thauer et al (11,12) showed that insensible water loss was similar in dogs and men. Since it is unlikely that much water would be lost from the interior of the follicles, because of their restricted openings, insensible water loss probably takes place from the surface epithelium and Thauer's observations indicate that the epithelia of dogs and men are equally permeable to water. It might be argued that the large range in thickness of the epithelium in different species makes an invariant barrier unlikely, but the stratum granulosum in which region the barrier is generally believed to lie (13) appears to be much less variable in thickness than other layers of the epidermis; it was found to be  $1\frac{1}{2}\mu$  thick in rabbits and  $2\mu$  thick in pigs. A mathematical treatment of the barrier (14) also indicated that its thickness varied little between species.

ACKNOWLEDGMENT

S. Peto of the Microbiological Research Establishment designed the statistical procedure described in Appendix B.

(Sgd.) M. Ainsworth,  
Supt., Protection Research Division.

RTT/MC

(Sgd.) W.S.S. Ladell,  
Assistant Director (Medical).

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STATISTICAL APPENDIX TO P.T.P. 726

A. Calculation of the average values and differences between groups

The analysis was performed on the original observations, not on the averages per experiment which are shown, for convenience, in the text. The means of the epidermal (E) and follicular (F) groups were found in the usual manner and the differences were calculated by taking all combinations of F-E within each experiment. An extract from the calculation of the difference between the number of follicles (n) covered with VX is given below:

Experiment	$n_F$	$n_E$	$n_F - n_E$
1	14	7	7
	19	9	12
	24		17
			5
			10
			15

The values of  $n_F - n_E$  thus obtained from the whole set of experiments were averaged; their internal variance was found and the 5% confidence limits for the mean calculated from it by means of a t-test. The same procedure was used for the penetration rate and penetration rate per unit area calculations.

B. Fitting to an equation

Equation 1 (Results, section 3) is a simple addition of the two routes of penetration:

$$np_F + Ap_E = R \quad \dots \dots \dots \quad (1)$$

For convenience in handling both sides were divided by  $Rp_E$  or  $Rp_F$ , to reduce the equation to the standard form  $y = mx + c$  :

$$A/R = -(p_F/p_E) \cdot n/R + 1/p_E \quad \dots \dots \dots \quad (2)$$

$$n/R = -(p_E/p_F) \cdot A/R + 1/p_F \quad \dots \dots \dots \quad (3)$$

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From the observed values of  $n$ ,  $A$  and  $R$  in each experiment 3-5 sets of values  $(n/R)$  and  $(A/R)$  were obtained. The regressions (a) of  $A/R$  on  $n/R$  and (b) of  $n/R$  on  $A/R$  were calculated, and the square root of the ratio of the regression constant for (a)  $(p_f/p_g)$  to that for (b)  $(p_g/p_f)$  was obtained; the two constants were combined with equal weight because  $n/R$  and  $A/R$  were known to contain comparable degrees of error.

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FIG.1. Metal restraining plate over pig flank skin.

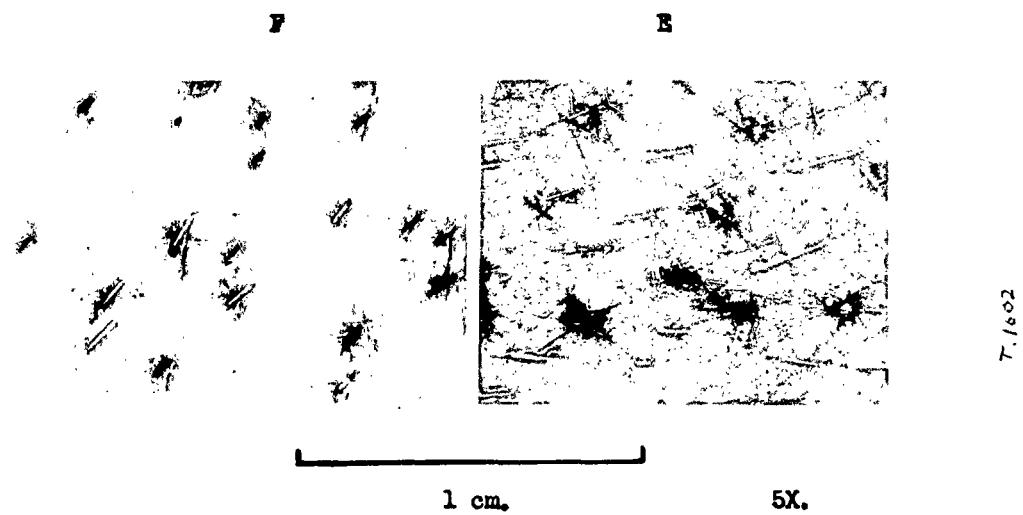


FIG.2. Dyed VX spots placed on ( F ) or between ( E ) the hair follicles.

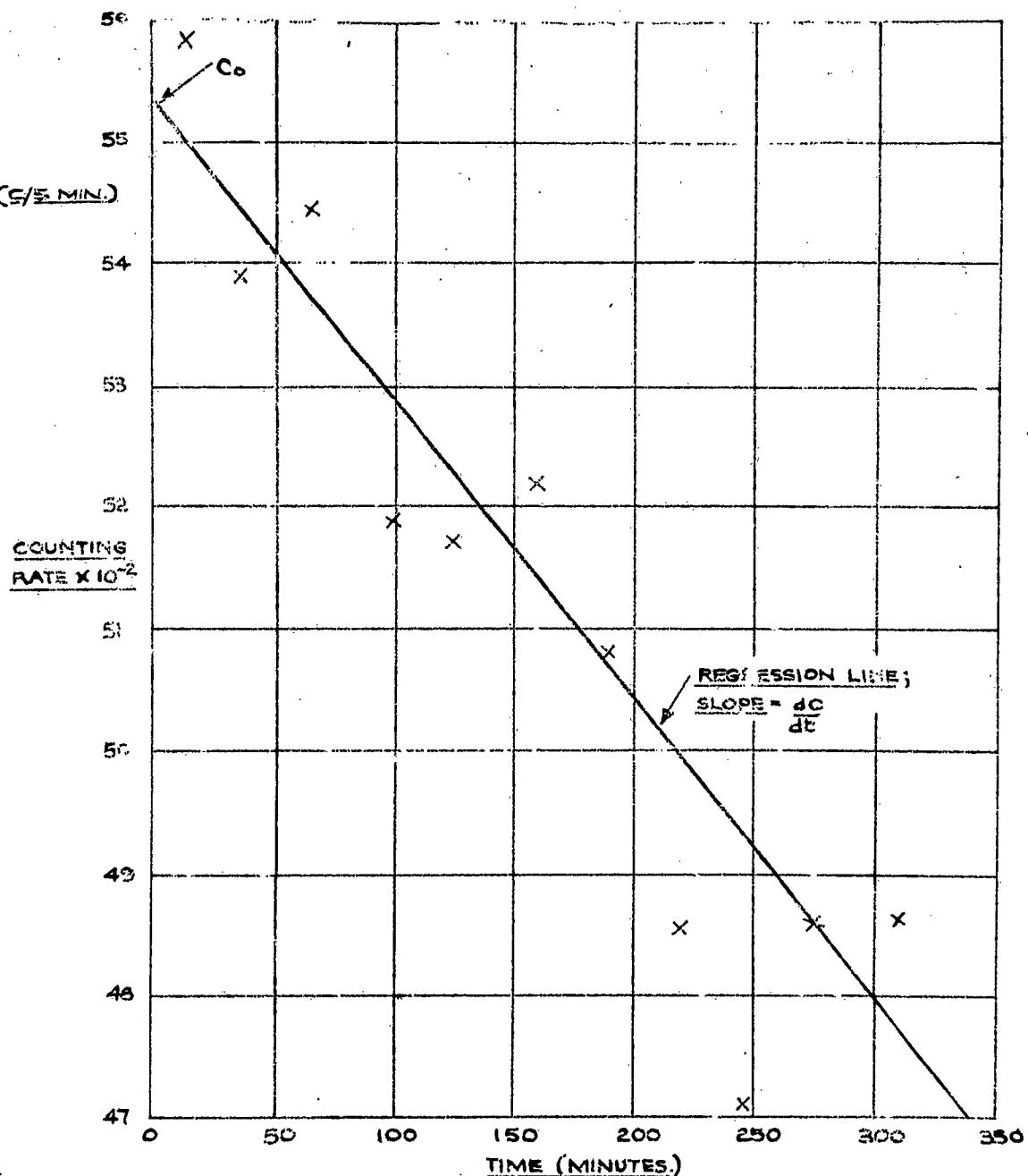


FIG. 3. THE REDUCTION IN RADIOACTIVITY ON THE SKIN AS PENETRATION PROCEEDS.

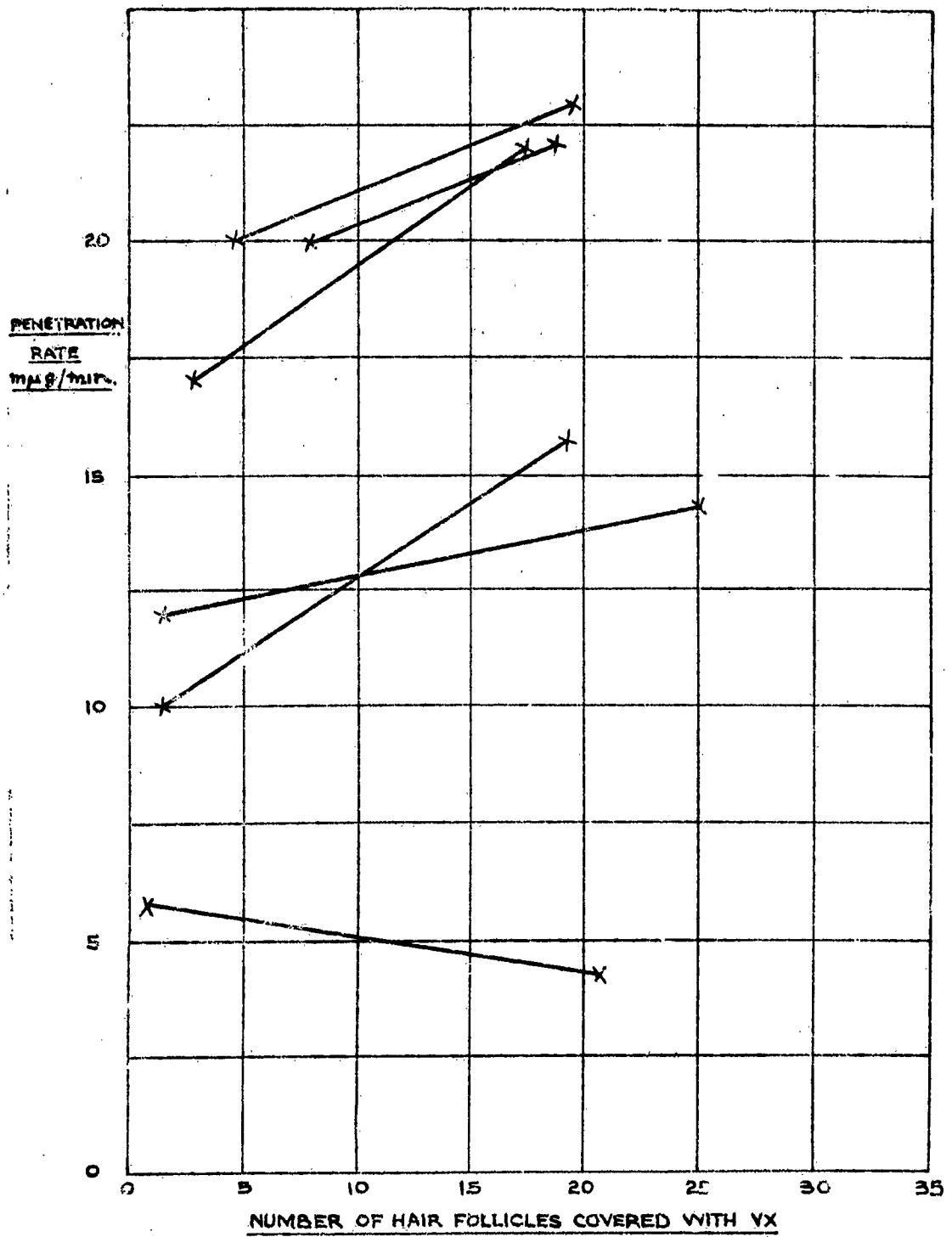


FIG. 4. THE EFFECT OF THE HAIR FOLLICLES UPON PENETRATION RATE.



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